SULFUR MUSTARD INHALATION INDUCED RESPIRATORY LESIONS IN GUINEA PIGS: PHYSIOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL STUDY

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ABSTRACT

Inhalation exposure to sulfur mustard (SM) vapor causes long term damage to the respiratory system. The lesions were characterized by specific physiological, biochemical and histopatholgical methods. Awake 128 guinea-pigs (GP) were exposed for 10 min to SM (1200-1700 µg x min/l). Respiratory parameters were monitored per animal before, during and after the exposure using plethysmographs. Biochemical and histological evaluations were performed at different time intervals for up to 7 days post exposure. SM inhalation resulted in a decrease in both respiratory rate and minute volume, and in an increase in tidal volume. These changes occurred immediately after the onset of exposure and lasted for up to 7 days. The changes in the respiratory parameters were accompanied by a massive reduction in O_2 diffusion capacity. Evaluation of bronchoalveolar lavage (BAL) fluid, indicated neutrophil infiltration, an increase in the protein content, and in the activity of both lysosomal enzymes and lactic dehydrogenase (LDH) in the alveolar space. In addition, a decrease in glutathione content was observed one day post exposure in the BAL fluid and the lung whereas an increase in lung glutathione content was observed 6 days later. Histological evaluation of the lungs and trachea revealed severe lesions in both tissues. Recovery was incomplete 7 days post exposure. The detailed characterization of the effect of SM inhalation offers a reliable model for the evaluation of potential therapies against SM exposure.

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INTRODUCTION

Sulfur Mustard is a chemical warfare agent that was first introduced during world war I, and more recently in the Iran-Iraq conflict (Freitag et al.,1991). Its dermal incapacitating effects were extensively studied and are well documented. However, the majority of SM casualties suffered from lung and airway injuries (Somani and Babu, 1989). The fatal cases and long term pathology were mainly due to respiratory complications. Yet, the literature lacks information concerning the characteristics and the mechanisms underlying pulmonary injury induced by SM inhalation.
Recently, we have established an experimental model of inhalation exposure to SM vapor in guinea pigs. The LCt_{50} of SM inhalation was determined at various time intervals post exposure for up to 30 days. LCt_{50} for 24hr, 8 days and 30 days, were determined at 2240, 1659, and 1578 µg x min/l, respectively.
In the current study we describe the effect of exposure to SM on the respiratory system of GPs, as evaluated by physiological, biochemical and histological methods.

METHODS

A total of 128 guinea pigs (300-400 grams), were exposed to SM inhalation in a controlled (temperature, humidity, air flow and concentrations) exposure chamber. Additional 60 control animals were exposed to air only. Groups of 4 GPs were exposed (head only) for 10 minutes to SM vapor (1250-1730 µg x min/l). Animals were restrained in an individual plethysmograph during the exposure and their respiratory rate and tidal volume were continuously monitored. The concentration of SM in the inhaled air was separately determined for each animal. This enabled the determination of individual SM dose.
At different time intervals throughout the 7 day study, animals were randomly assigned to physiological, biochemical and histological evaluation .
Physiological evaluation included clinical observations and monitoring of animal body weight. Respiratory parameters were measured and oxygen diffusion capacity in the lungs was assessed by measuring the changes in arterial oxygen pressure during pure oxygen inhalation.
Bronchoalveolar lavage was performed on anesthetized animals using routine procedures (Henderson, 1988). Saline was introduced into the lungs (2x3ml) and the recovered fluid was centrifuged. Pelleted cells were differentially counted on Giemza stained slides. In addition, the supernatant fluid was used to determine the following parameters: protein, lactic dehydrogenase activity (LDH), acid phosphatase activity (ACP), BAL fluid procoagulant activity (Idell, 1989) and glutathione (GSH) (Anderson, 1985). Following the BAL, the lungs were perfused, flash frozen and kept at -80°C for further biochemical analyses, e.g. GSH content, dry weight.
Histological evaluation of the lungs and trachea was performed on Hematoxylin & Eosin stained paraffin sections and included microscopic and morphometric assessments, utilizing a computerized image analysis system (Galai, Israel). The cellular volume (cells and edema products) in the lung sections was used as an indicator for inflammatory response and

alveolar consolidation.

RESULTS

Physiology

Inhalation exposure to SM caused significant, dose dependent decrease in body weight. The decrease was more severe in the animals exposed to the higher concentrations of SM (1250 vs 1730 µg x min/l), with no recovery by the end of the week (Fig. 1).

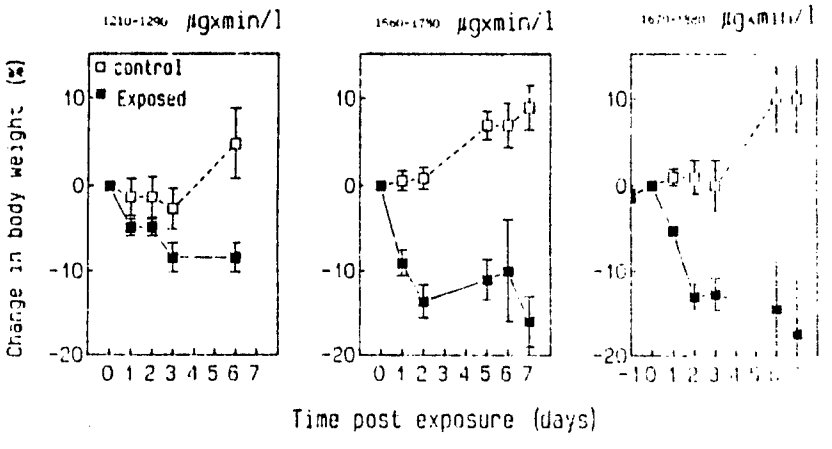

Figure 1: The effect of SM inhalation on guinea pigs growth:
Body weight changes at various times post-exposure to SM versous body weight changes of control animals.

A significant decrease in respiratory rate and in minute volume and an increase in tidal volume were apparent in the exposed GPs. These changes occurred immediately after the onset of exposure and lasted in most cases throughout the observation period.
The changes in the respiratory parameters were accompanied by a massive reduction in the diffusion capacity of O_2 in the lung. Partial recovery of this capacity was seen 24-48 hrs post exposure with no further improvement for the rest of the study.

Biochemistry

Sulfur mustard inhalation caused alterations in several parameters measured in the BAL fluid. The degree of lung damage and the number of animals affected were found to be dependent on the SM concentration in the inhaled air. At 1250 and 1730 µg x min/l, 15% and 85% of the GPs (respectively) had lung injuries as defined by significant changes in the following biochemical parameters: An increase in the percent of neutrophils was observed as early as 5 hrs post exposure and remained at a high level throughout the experiment (Fig. 2). Concomitantly, a large increase in the concentration of protein and in the activities of ACP and LDH were detected. The only parameter that showed partial recovery toward the end of the observation period was the protein concentration in the BAL fluid.

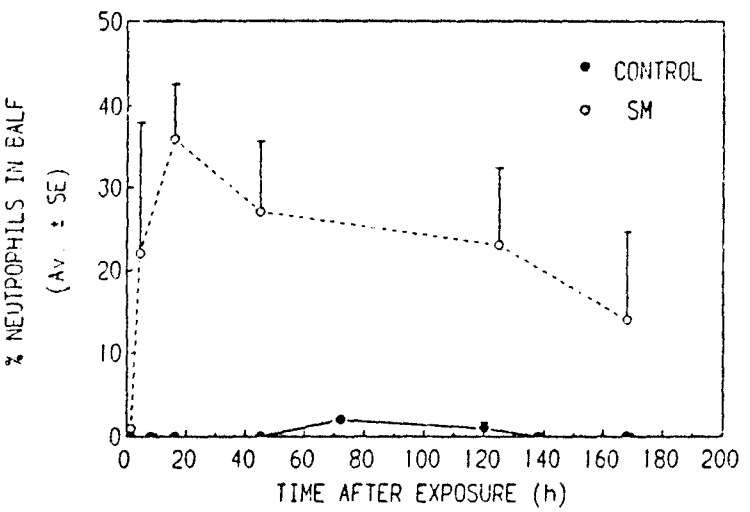

Figure 2: The effect of SM inhalation on BALF neutrophil level
Neutrophil number in the BALF of GP at various times after exposure to SM (1730 µgxmin/L) versus controls.

Twenty four hrs post exposure, an infiltration of erythrocytes into the alveoli was observed accompanied by a decrease in the number of alveolar macrophages. Throughout the week erythrocyte levels remained high while the macrophage number recovered. These findings were concomitant with a significant increase in lung dry weight in the most affected GPs.
Total GSH was assessed both in the BAL fluid and in lung homogenate. A decrease in the GSH was seen both in the BAL fluid and in the lung homogenate 24 hrs post exposure. This was followed by an increase in lung GSH (but not in the BAL fluid) toward the end of the week.

Histology

Respiratory lesions were already detectable 1 hr post exposure and were characterized by intra-alveolar edema, loss of cilia and increased mucous secretion. An ongoing deterioration of the respiratory system, with a maximum between 12 to 48 hrs was observed. At this time, vesication and necrosis were noted in the tracheobronchial tree and the edema extended to most parts of the lung (Fig. 3).

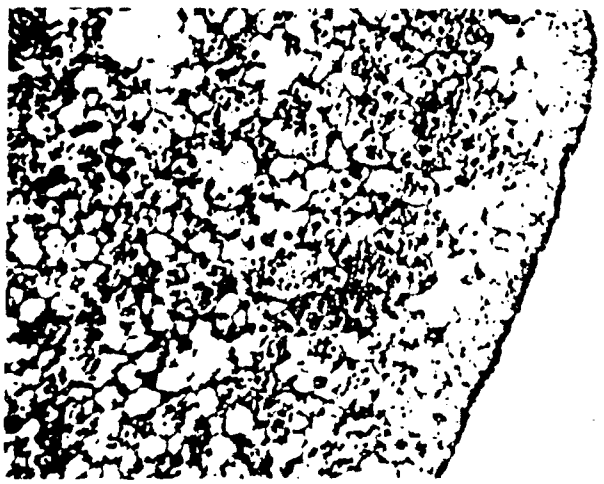

Figure 3: Light micrograph, showing lung damage (edema and cellular infiltration) at 48 hrs post SM inhalation in guinea pigs.

Thereafter, infiltration of inflammatory cells and cellular thickening of alveolar septa took place. Although healing processes were noted, only partial recovery was seen after a week and the animals continued to suffer from lung and airways injuries.

A morphometric analysis representing the time course of lung injury is shown in Fig. 4. Morpho-physiological correlations revealed that the extent of the damage in the lung correlates with oxygen diffusion capacity ($r = -0.86$).

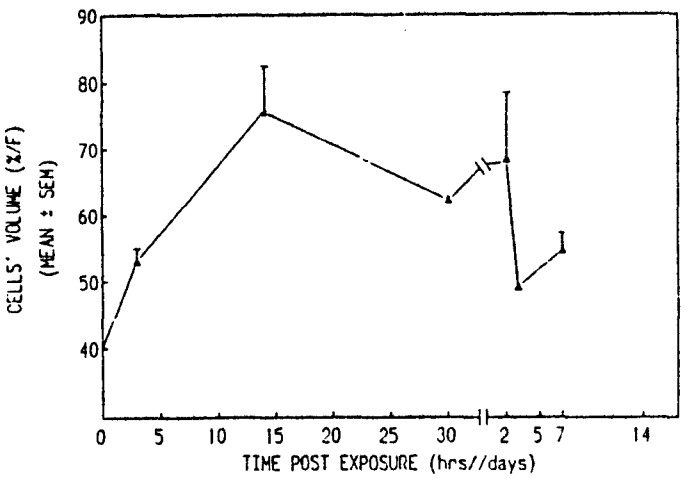

Figure 4: Morphometric analysis showing the extent of lung injury following SM inhalation in guinea pigs. Lung's surface coverage (% frame) was measured in 3 serial sections from each animal.

DISCUSSION

The results presented here characterize the damage induced in the respiratory system by SM inhalation. The time course of the impairments following the SM exposure is described.

Respiration rates and body weights were found to be highly sensitive parameters for the clinical evaluation of SM intoxication. An immediate and intense decrease in the respiration rate was apparent at the onset of SM inhalation. This lead to the decrease in the minute volume. The alterations in respiratory parameters, together with a massive edema were probably the cause for the long term and marked reduction in the diffusion capacity of O_2 in the lung.

The cascade of events leading to the respiratory lesions following SM inhalation is initially detected as a damage to the respiratory mucosa and as alveolar edema followed by a chronic inflammatory response in both lung and tracheobronchial tree.

Damage to the alveocapillary barrier was evident 5 hrs post exposure as indicated by the presence of erytrocytes and by the high protein levels in the BAL fluid. The protein level in the BAL reached a maximal value at 24 hrs post exposure. These findings coincides with the lowest levels of pO_2 measured.

A cytotoxic effect of the SM exposure on the lung, was evident by the decrease in number of macrophages at 24hr, and by the continuous high levels of LDH activity.
Other parameters, such as GSH levels, lung dry weight etc. demonstrated lower sensitivity to SM inhalation, but may help to elucidate the mechanisms underlying the SM intoxication.
The range and variety of our experimental procedures will enable precise evaluation of potential treatments against alkylating agent lesions in the respiratory system.

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